Enabling Single-Software Multiple Attribute Methodology (MAM) for Assessment of Biopharmaceutical Product Quality Attributes

Application of BioPharmaView™ 3.0 Software for a Streamlined MAM Workflow

Zoe Zhang[2], Fan Zhang[2], Sean McCarthy[1]
[1]SCIEX Framingham, MA (USA), [2]SCIEX Redwood City, CA (USA)

Introduction

Development and production of biopharmaceuticals is complex. Even minor impurities, or changes in attributes such as glycosylation or charge heterogeneity, can have a profound impact on the safety and efficacy of the final product. Traditionally, multiple analytical techniques have been required to assess the full range of biopharmaceutical product attributes. But the inevitable consequence of multiple analytical techniques is greater expenditures of time and resources.

The Multiple Attribute Methodology (MAM), an orthogonal approach based on peptide map separation coupled with high-resolution mass spectrometry, is rapidly emerging as a powerful tool for characterization and monitoring of biopharmaceutical attributes. The range of attributes that can be monitored using this approach is extensive. MAM can be used to assess, track, and provide detailed data on multiple, specific biologic product quality attributes at the peptide level (Figure 1). In addition to tracking the therapeutic molecule itself, MAM can be used to detect known impurities related to production of the biotherapeutic, as well as unknown impurities (new peaks) present in samples but not in corresponding standards.

Essential to successful implementation of an MAM workflow is software that can manage all aspects of the workflow, including: product quality attribute (PQA) definition, tracking, and quantification; detection of known and unknown impurities; and reporting. This technical note describes the use of SCIEX BioPharmaView™ 3.0 Software for MAM workflow management. BioPharmaView software can manage all aspects of an MAM workflow from a single project. It eliminates the unnecessary complexity that comes with using multiple software packages.

Key Features

- BioPharmaView software provides a single-software solution for MAM workflows, in addition to traditional core characterization workflows, such as intact mass analysis, subunit analysis, and peptide mapping

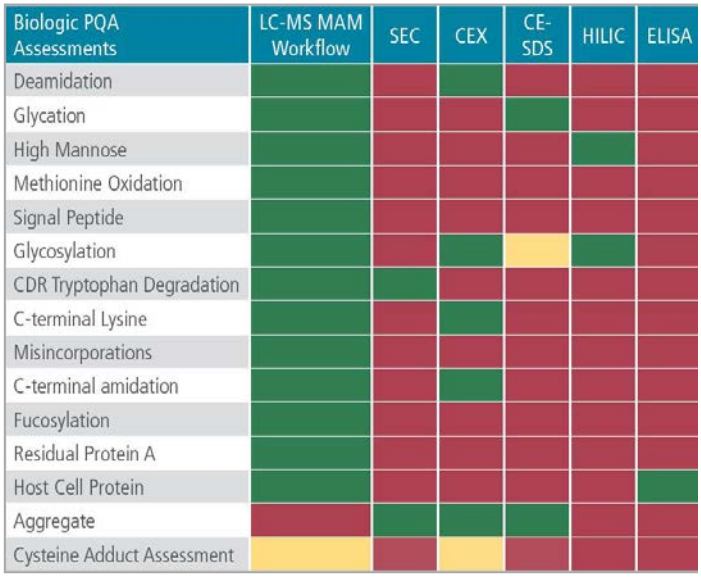

Figure 1. Comparison of traditional assays to and accurate mass LC-MS MAM assay for a selected set of biotherapeutic attributes

- Simple method creation on a single platform
- Powerful product characterization, attribute definition and tracking, and quantitation
- Flexible custom calculations for attribute-level assessment based on specific user needs
- Reliable detection and monitoring of both specified and unspecified impurities
- Concise review and reporting of targeted attributes

Method Creation

Creation of a multiple attribute assay in BioPharmaView software is simple and streamlined. The assay starts with the definition of the target protein sequence. If multiple chains are required, each is defined separately as shown in Figure 2. Known disulfide linkages and any modifications that may be present are applied, entered, and positioned to specific amino acids within the sequence. Desired modifications not built into BioPharmaView software can easily be added. These custom modifications are then available for use across all BioPharmaView projects.

In addition to the target biotherapeutic, any known impurity sequences are entered separately as a targeted peptide or protein sequence. Impurity sequences are treated identically to the target molecule during in-silico digestion, using the parameters defined for the biotherapeutic. Impurity sequences are searched and presented separately throughout the workflow for easy distinction from the biotherapeutic.

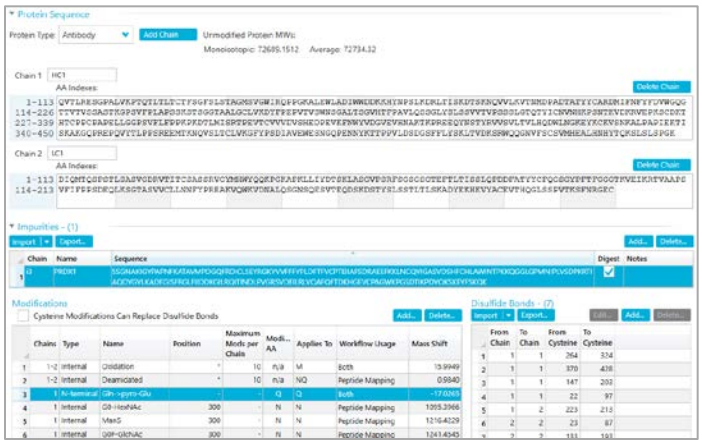

Figure 2. Definition of protein sequence, disulfide bonds, and modifications within BioPharmaView software

The assay information is completed by definition of digestion parameters using a range of built-in cysteine alkylation reagents and digestion enzymes. The maximum number of modifications and missed cleavages to search within the data is also defined, as shown in Figure 3.

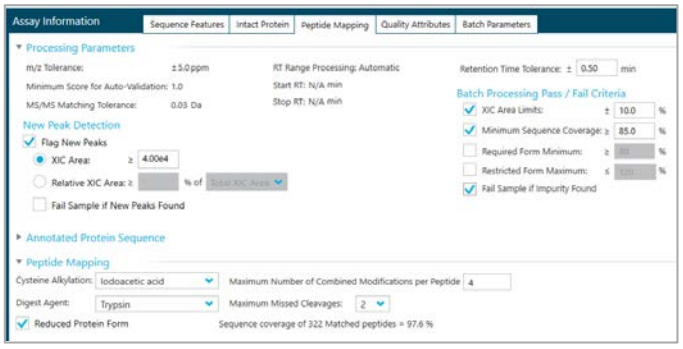

Figure 3. Definition of search parameters and definition of New Peak Detection criteria for batch analysis.

Characterization

Using the defined assay information, acquired data is submitted for processing. Characterization of samples is accomplished automatically by the BioPharmaView software. Peptide assignments are based on defined search parameters by correlation of MS- and MS/MS-level data. After processing, peptide results are easily reviewed using a single interface. To expedite review, results are easily sorted using a wide range of available filters.

Peptide modifications defined in the assay are automatically annotated in the peptide results and are easily filtered to expedite review. In cases where modifications are not automatically positioned, assignment of the position is guided using pre-populated scoring results from processed data. When a modification has been positioned, the position information is used in ongoing studies. After characterization is complete, the assay information is updated for use in batch analyses.

Attribute Definition

Targeted attributes are easily defined within BioPharmaView software as shown in Figure 4. Applying the same filter criteria used in characterization, targeted attributes can be compiled in peptide sets. Each attribute is captured within its own peptide set, which contains all of the data that matched the defined filter criteria. The attribute peptide sets are named and can be shared within and between projects. Sharing peptide sets reduces the overall time required to define assays and may reduce variability in set definition.

Using the defined peptide sets, attribute levels are calculated using a highly flexible custom calculation engine. Use of custom calculations enables users to define how each attribute level will be determined, as shown in Figure 4. As each attribute is defined, the calculated values are added to the assay displayed within a table. There is no practical limit to the number of attributes that

can be defined or monitored as part of an assay, providing the flexibility to monitor a large number of attributes simultaneously.

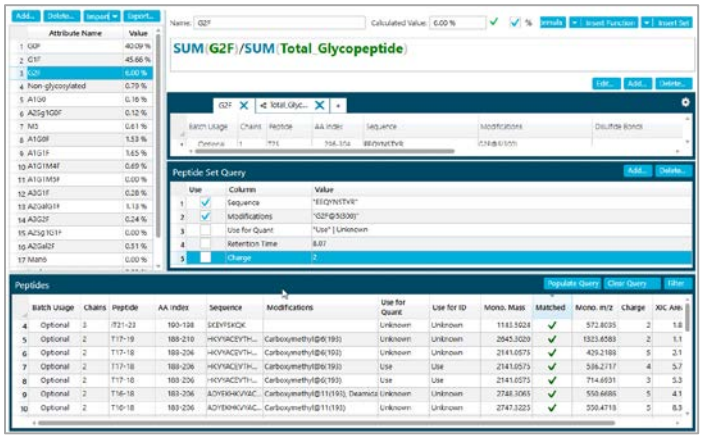

Figure 4. Definition of quality attributes and custom calculations within BioPharmaView software

For each attribute defined in an assay, acceptance criteria are also defined. The range of values that are acceptable for each calculated attribute response is defined independently. Ranges may be set based on a percentage deviation from a defined value or as needed to be greater/less than a specific value. The defined ranges are used to determine overall pass/fail status of their corresponding attributes.

Attribute Quantification

Sample batches are submitted for processing in BioPharmaView software, which tracks defined attributes across submitted sample sets. Batch analysis can be performed on TOF-MS data or SWATH® acquisition data. SWATH acquisition provides greater detail by generating MS and MS/MS data for all detectable components in each sample. The additional fragment data provided by SWATH acquisition enables identification of detected new components without the need for reanalysis.

Processing a batch of two or more data files enables the comparison of samples processed in the batch. For each selected attribute in the assay, a concise summary is presented within the Attribute tab. This tab provides an overview of the calculated attribute levels for each sample, a pass/fail indication, and the range over which the attribute will pass (Figure 5).

Each attribute can be selected independently to view the underlying data used for each calculation. Selection of dentified species within the peptide results provides a view of the MS and, if acquired, MS/MS data for the selected component. Data for matched peptides not included in the attribute method are accessed in the Matched tab. Investigation can be completed on each sample individually or by comparison between two or more samples.

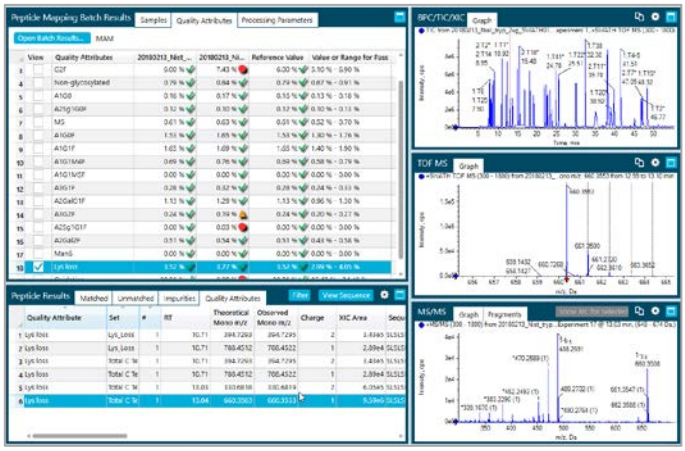

Figure 5. Review of batch analysis results in BioPharmaView software. Results for defined attributes are presented for review with pass fail indication and acceptance ranges. Supporting data is easily accessed in the same window.

Monitoring of Known Impurities

When defined in the assay, known impurity results are presented in the Impurities tab of the peptide results (Figure 6), separate from the defined therapeutic. Each impurity is clearly indicated with distinct nomenclature and indication of which peptides were identified.

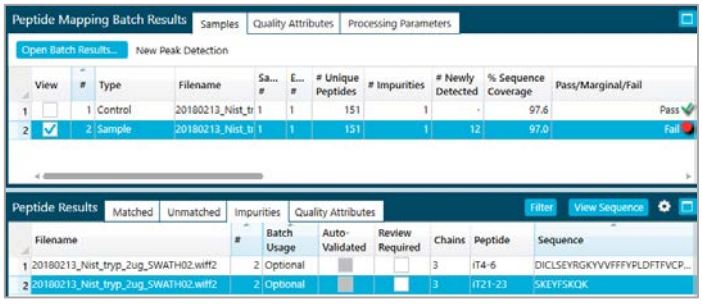

Figure 6. Detection of defined impurities. Impurity information is provided in a separate tab and clearly indicated as impurities.

New Peak Detection

BioPharmaView software supports new peak (unknown impurity) detection during batch analysis if selected within the assay. The detection of new peaks is performed by comparing each sample with a control. It is important that both the control and sample are prepared and analyzed as part of the same study to account for

variability in sample preparation. To execute new peak detection, the threshold for detection is defined, guided by ongoing characterization work. It is possible to define the threshold based on absolute or relative signal intensity (Figure 3). Samples may be automatically failed if new peaks are detected, even if all other attribute parameters pass.

When executing new peak detection in batch analysis, one of the data files must be defined as the control. The control sample serves as the benchmark against which the other samples that are compared. Often, the control sample is one that has been previously characterized and is well understood.

New peak detection results are presented in a concise summary within the batch results. As shown in Figure 7, the overall number of peptides for each sample is listed, as well as the impurities detected. The number of new peaks detected is also provided in the results.

If further interrogation of new peaks is required, peptide results are easily filtered to display only those components which are flagged as newly detected. For detailed information on new peaks, each can be selected and the corresponding MS and MS/MS spectra viewed. If a new peak has been seen previously in characterization efforts, or is not a specific concern, its designation is easily changed. Changing the status of a new peak requires a justification for the change to be provided. This justification is captured as part of the assay. Detection of a new peak can also cause the sample to automatically fail, as evidenced by the red dot for the sample shown in Figure 7.

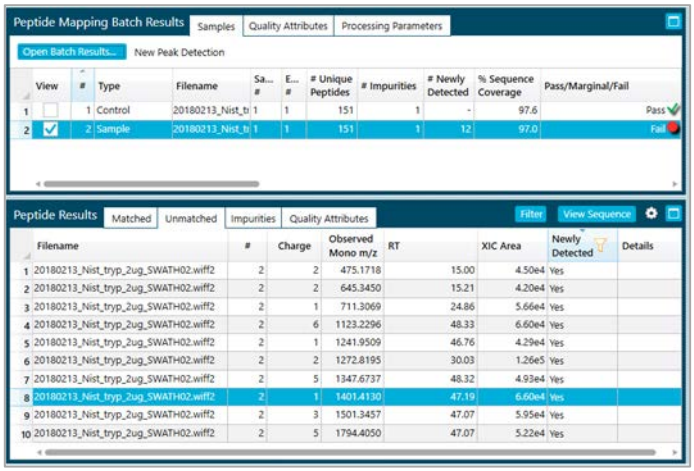

Figure 7. Results for new peak detection within BioPharmaView software. The number of new peaks, as well as the data supporting their detection, is easily displayed.

Reporting

Results from batch analyses are compiled in a concise report which includes assay information and processing parameters as well as a summary table of attributes defined in the assays. The report template is a standard template within the software. Within the report, each attribute is flagged as to whether it has passed or failed. The number of impurities and new peaks detected is also summarized. For every sample, an overall pass/fail indication is included to expedite review.

Conclusion

The BioPharmaView software provides a single software package for automating a complete MAM workflow, including: characterization, attribute definition, custom calculations, known impurity detection, unknown impurity (new peak) detection, and reporting. The ability to complete the entire workflow within a single software solution reduces the effort and eliminates potential transcription errors associated with the use of multiple software solutions. Taken together, BioPharmaView software provides a superior solution for the development and execution of MAM assays.

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Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
Phone 508-383-7700
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